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BULLETIN No. 4 OF THE HYGIENIC LABORATORY,
*Treasury Department, U. S. Marine-Hospital Service,*
WALTER WYMAN, *Surgeon-General.*

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# VIABILITY

# OF THE

# BACILLUS PESTIS.

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WASHINGTON, D. C.

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# LETTER OF TRANSMITTAL.

TREASURY DEPARTMENT,
MARINE-HOSPITAL SERVICE,
*Washington, April 25, 1901.*

SIR: In May of last year a preliminary note upon "The Viability of the Bacillus Pestis" was submitted and published in the "Public Health Reports," Vol. XV, No. 21.

The work has been continued without interruption in this laboratory since that time and I have now the honor to make a more complete report upon this important subject.

During my absence in Europe this work was continued by P. A. Surgeon H. D. Geddings, M. H. S., especially in relation to food stuffs, and his report is embodied in his own words.

I have also to acknowledge material help from Assistant Surgeons Currie and Long in carrying on many details of the work while they were stationed in the Laboratory.

Respectfully, M. J. ROSENAU,
*Director, Hygienic Laboratory.*

The SURGEON-GENERAL,
*U. S. Marine-Hospital Service,*
*Washington, D. C.*

# THE VIABILITY OF THE BACILLUS PESTIS.

[By M. J. Rosenau, Passed Assistant Surgeon, Director Hygienic Laboratory, Marine-Hospital Service.]

There are many factors that influence the life of the bacillus pestis in the outer world. Some of these influences, such as temperature, moisture, and light, are well understood. The bacillus withstands drying very badly. It can not live long in the direct, warm sunshine. High temperatures are invariably fatal.

In the light of these experiments and the published work of others we have to modify considerably our views upon the viability of the bacillus pestis. It does not always and under all circumstances die in so short a time as a few days, as the work of Kitasato and Wilm first indicated. We now know that the organism may live for months, and even years, in a test tube, on a moist albuminous medium. And the present work shows that even when dry it may live over four months, provided the temperature is cool—less than 20° C.

The bacillus of plague can in no sense be considered a tender organism, as was at first supposed. It is much easier to cultivate than the lanceolate coccus of pneumonia or the pathogenic streptococci. In this respect it resembles more closely the hardier of the hemorrhagic septicæmic group.

In this laboratory we have worked with the organism from seven sources: from Djiddah, Oporto, Rio de Janeiro, Bombay, the New York Quarantine case, Glasgow, and San Francisco. We find that all these seven varieties thrive well on artificial media under the usual laboratory conditions.

We tested the life history of this organism upon a great variety of objects and under various conditions. We attempted to imitate nature. But we can not imitate all the conditions under which the organism may exist in nature, and we ought not, therefore, to apply the experience of the laboratory too literally to the life history of the plague bacillus outside of the body. We may determine with fair certainty the length of time the bacillus may live under given conditions. But these conditions are more or less arbitrary, and to a certain extent artificial. In general terms, we can state whether it is a hardy organism, resistant to influences usually detrimental to bacterial life, or one that loses its virulence and dies quickly when removed from its natural habitat.

The bacillus of plague does not exist in nature on sterile glass cover slips, nor yet in the desiccator over concentrated sulphuric acid, which were conditions used by some authors who have reported their results on this question.

It is also necessary to call attention to certain conditions which obtained in most of these experiments, that are very far from the usual state of affairs.

The test objects were very abundantly inoculated with a pure culture of the bacillus pestis of known activity and virulence. Often the test objects were saturated. The cultures had been grown for a long time upon artificial media in the laboratory, so that their vitality was probably strongly influenced. It is a well-known fact that virulent pathogenic bacteria may at first grow very poorly upon the ordinary laboratory media, but by successive cultivation they become accustomed to the new conditions, so that they finally thrive abundantly; that is to say, they take on a sort of saprophytic existence. Such cultures would doubtless resist the various influences to which they are exposed in the laboratory tests better than another race direct from the blood or tissues. In fact, it is found that the plague bacillus in the blood and tissues from a rabbit usually dies out rather quickly when dried upon the test objects. On the contrary, bouillon cultures dried on similar objects and under similar conditions live a much longer time.

Another departure from normal conditions was the fact that all the test objects receiving the abundant inoculations of the virulent pure cultures were sterile. In other words, not only were cultures of the bacillus used that were accustomed to a saprophytic existence, but these cultures were placed upon sterile test objects and protected against contamination, so that they were relieved from that microbial symbiosis which, in the economy of nature, plays so important a part in the suppression of pathogenic micro-organisms. It is known that in organic mixtures the hardier saprophytes tend to overpower the bacillus pestis. This even occurs to a certain extent in the blood and buboes of patients sick of the disease, as well as in dead organic infusions. The bacillus pestis is not known to have synergists among the lower forms of life. But that such exist may well be inferred from analogy.

Further, different races of the plague organism have different powers of resistance to unfavorable influences. Some races of plague are hardier than others, just as some races of mankind are hardier than others. Batzeroff states, "There undoubtedly exist varieties that attenuate very quickly and die outside the living body in a relatively short time. There are other varieties that retain their viability under similar conditions a long time." Bacteria show quite as strong a tendency to adaptability as the higher animals. They may become accustomed to influences by a gradual survival of the fittest so that they finally resist conditions that would be fatal to the parent stock.

All these facts must be borne in mind in drawing conclusions from laboratory experiments.

## PLAGUE AND FOOD.

Our experiments show that food products may harbor the infective principle of plague, but according to experience food products are not much to be feared as far as their probability of carrying the infection is concerned. This latter statement does not apply to milk and its products, for milk is a good culture medium for the bacillus pestis; and we kept it alive seventeen days in cheese and seventy-two days in butter.

On the surface of food products it usually died very quickly. It did not live twenty-four hours on orange peel. We had similar results with figs and raisins and a large quantity of Chinese food products, such as smoked and dried ducks, dried oysters, dried cuttle fish, dried ducks' gizzards, ducks' gizzards dried and placed in oil, smoked and dried pork, and duck eggs preserved in a mixture of mud and rice chaff, all of which were infected with the bacillus pestis and kept at 37° C.

In rice we found it alive eighteen days after inoculating.

These results correspond with all our other experiments, which plainly prove that the bacillus can not live long on the surface of objects, when dry, at temperatures above 30° C. These results are also in accordance with the clinical and pathological facts, for cases of plague that have their origin in the alimentary tract are comparatively rare, and the spread of the disease has not, as far as I am aware, ever been clearly attributed to food products.

However, tonsillar and intestinal forms of the disease have been recorded, and these cases may be due to the fact that the bacillus can live in milk, butter, and cheese, and similar moist albuminous media, as well as in water (*vide infra*) a long time.

## PLAGUE AND WATER.

Plague is certainly not a water-borne disease, although we find that the plague bacillus can live a long time when abundantly inoculated into water containing a trace of organic matter. In one case we kept it alive one hundred and sixteen days, and in another ninety-six days, in water preserved at low temperatures, 17° to 19° C. Under the same conditions the organism lived only six days at 37° C.

In these experiments the water was sterile and abundantly inoculated with a vigorous culture and carefully preserved from contamination in the dark. In nature the water certainly would not be sterile, the effect of sunlight and dilution would play an active part to diminish its vitality and infective power.

### THE EFFECT OF TEMPERATURE.

The effect of temperature upon the bacillus pestis is very remarkable. That it is very sensitive to slight changes is well shown in Table A. In fact, temperature seems to be the most important factor in the viability of the organism. It may be kept alive and virulent a very long time in the cold, even though dry, but it can not live long when dry at the temperature of the body. High temperatures, such as 70° C. or more, are invariably fatal in a few minutes. All the published experiments agree upon the influence of temperature upon this microbe. It was this that led some of the early workers to conclude that they were dealing with a frail organism. It is frail when dried at 37° C., but may live for months in the cold. We have never been able to keep it alive more than a few days when dry at 37° C.—three days in flannel, two days in sponge. On the contrary we had little difficulty in keeping it alive on a variety of objects three and four months at 17° to 19° C.

The bacillus is not as sensitive to temperature when kept moist, for under such conditions it will live a very long time in albuminous media at 37° C.

### PLAGUE AND COLD WEATHER.

From the experimental studies with the plague bacillus we would infer that the endemic foci of plague should be in cold climates. The organism dies so rapidly when dried at temperatures above 30° C. that we would not expect the disease to be very tenacious in the Tropics. This supposition is only partially borne out by the facts. A certain number of the endemic foci of plague are in a very rigorous climate. Such in particular are the regions of the Garwhal and of Kumaon,[1] where the disease came from the snow-capped Himalayas. In the Transbalkan districts the average temperature in winter is −20° C. In Mongolia the climatic conditions are similar. At Vetlianka during the epidemic of plague in 1878–79 the temperature was −12° C.

### PLAGUE IN THE TROPICS.

Netter states in his "La Peste et son Microbe" that the disease has never invaded the regions where the temperature is tropical, and he notes its disappearance in Egypt with the summer. This statement would be a confirmation of the fact that the organism does not as a rule live outside of the body very long at temperatures of 37° C. and over.

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[1] Netter, "La Peste et son Microbe."

But there are other ways of spreading the disease that must be taken into account, such as the direct contagiousness of the pneumonic form of the disease through the coughing, etc., of the plague-laden sputum; also by the means of fleas and rats. This may account for the existence and spread of the disease in such hot countries as the Bombay Presidency, Manila, the Island of Mauritius, Rio de Janeiro, and Santos, all of which are in the Tropics and suffer with tropical heat.

### THE EFFECT OF MOISTURE.

Moisture is a definite factor in the viability of the bacillus pestis. The organism must have moisture to grow, and it may remain alive and virulent a very long time in the presence of moisture.

It usually dies quickly when dry. However, this is not invariably the case. We have been able to keep it alive in media such as dried albumin for one hundred and twenty-five days, when it was still virulent for mice. But to be kept alive when dry the organism must be cold, i. e., exposed to a temperature less than 20° C.

In no instance could the organism be kept alive when dry at a temperature of 37° C. for more than a few days.

### EFFECT OF SUNLIGHT.

Our experiments confirm those of other workers in this field, who find that for the most part the bacillus pestis soon dies when exposed to bright sunlight. Our work leads us to the conclusion that the heat as well as the sunlight plays an important rôle; also that the effects of the sunlight do not penetrate very deeply. It is therefore safe to say that objects may be efficiently disinfected on the surface by exposing them all day to a bright sun, provided the temperature in the sun is above 30° C.

### PLAGUE IN THE SOIL.

The plague bacillus was kept alive a long time in moist garden earth, especially when kept cool. It dies very quickly in dry earth. We were not able to keep it alive longer than twenty-four hours at any temperature in dry earth.

As moist earth will preserve the life of the bacillus it is easy to understand how the infection may live in dirty dwellings. It requires no stretch of the imagination to understand how the infection may be conveyed by the dirt and dust of moist, sunless habitations.

### PLAGUE ON NEW MERCHANDISE.

We have not succeeded in keeping plague alive very long when dried upon the surface of objects; even on plush, carpet, paper, wood, sawdust, bone, etc., it usually dies within a few days. In porous substances such as sponge we found it alive after one hundred and twenty-five days, when allowed to dry, at 19° C. Here again temperature plays an important rôle, for at 37° C., all the other conditions being the same, it lived only two days.

It is therefore very unlikely that new merchandise would be so contaminated as to carry the infection of the disease, especially in hot weather.

## PLAGUE IN CLOTHING AND BEDDING.

The bacillus of plague lives long in albuminous matter. Clothing and bedding are especially apt to be contaminated with the discharges from buboes and blisters, sputum, etc. Articles so infected and kept in a cool, moist place could retain the active infective principle a very long time. Our work shows plainly that clothing and bedding may harbor the bacillus of plague for months. In one instance we kept it alive on a piece of crash ninety-seven days; in albumin gelatin balls one hundred and twenty-five days; in sponge, also, one hundred and twenty-five days; in wool fifty-two days.

## PLAGUE IN THE MAIL.

According to our results the plague bacillus can not live long in letter mail. In seven tests made with cultures of the organism on paper we found that it usually died within twenty-four hours. At most it kept alive eight days on paper allowed to dry, and fourteen days on paper kept in a moist atmosphere. To live this long it must be kept cool, for, just as in all our other experiments, it died very quickly when dried at the body temperature. We had similar experiences with plague blood upon paper. Letter mail is therefore not apt to carry the infection of plague a long time, especially in warm weather.

## VIABILITY AND VIRULENCE.

The bacillus pestis often loses its virulence before it dies. In many of our experiments we found that the time came when the organism grew in bouillon, but lost its pathogenity for animals. This is an important fact from an epidemiologic standpoint, for an attenuated plague bacillus is probably harmless to man, even though its virulence can be increased by artificial means in the laboratory.

## GASEOUS DISINFECTANTS FOR THE PLAGUE BACILLUS.

The experiments conducted in this laboratory plainly prove that either sulphur dioxide, when moist, or formaldehyde will kill the bacillus pestis when applied in the strength and methods usually employed for these gases as disinfecting agents. In order to be effective there must be direct contact between the gas and the germ. In other words, these gaseous disinfectants can only be depended upon as surface disinfectants.

In Laboratory Bulletin No. 3. on "Sulphur dioxide as a germicidal agent," Geddings found that 4.25 per cent of that gas for twenty-four hours' time invariably kills the bacillus pestis on the surface.

My own work with formaldehyde (soon to be published) shows that the plague organism is as susceptible to this germicidal agent as other non-sporebearing organisms. Any of the standard methods for evolving the free formaldehyde gas are applicable.

As far as practical disinfection for plague is concerned, it may be mentioned here that sulphur dioxide is probably a much more useful agent for use in ships, stores, houses, and dwellings infested with vermin, because it is destructive to the higher forms of animal life, whereas formaldehyde fails to kill mammals and insects with the same certainty that it kills microbes. In combating plague it is very important to kill the fleas, rats, mice, and other forms of animal life capable of carrying the infection. Sulphur has this power, which formaldehyde totally lacks.

## CHEMICAL DISINFECTANTS FOR THE PLAGUE BACILLUS.

The bacillus of plague shows no special peculiarity in its relation to chemical disinfectants in solution. We have found the following ordinary disinfectants to have very marked aseptic powers.

*Solutions which prevent the growth of the bacillus pestis.*

| | |
|---|---|
| Bichloride of mercury and hydrocloric acid | 1 to 50,000 |
| Bichloride of mercury | 1 to 25,000 |
| Formalin | 1 to 25,000 |
| Carbolic acid (pure) | 1 to 830 |

The following table gives the results of the German plague commission upon the power of disinfecting agents in relation to the bacillus pestis:

| Agent. | Number of minutes necessary. |
|---|---|
| Carbolic acid: | |
| 5 per cent | 1 |
| 1 per cent | 10 |
| Lysol: | |
| 2.5 per cent | 1 |
| 1 per cent | 5 |
| Sublimate, 1 to 1,000 | Immediate destruction. |
| Chloride of lime, 1 to 1,000 | 15 |
| Quicklime | 30 |
| Milk of lime mixed with equal quantity of salt | 60 |
| Black soap: | |
| 1 per cent | More than an hour. |
| 3 per cent | 30 minutes. |
| Sulphuric acid, 1 to 2,000 | 5 |
| Hydrochloric acid, 1 to 1,000 | 30 |

## THERMAL DEATH POINT.

We had abundant opportunity in our work in making Haffkine prophylactic in demonstrating that a temperature of 70° is invariably fatal in a short time.

The following table shows the maximum number of days we have kept the organism alive on the objects used in these experiments, and the temperatures under which the experiments were conducted. The table shows at a glance how frail an organism the bacillus pestis is at the body temperature. At 22° to 29° C. it shows much more resistance. At 17° to 19° C. it lives a very long time, even when dry.

## TABLE A.

*Maximum number of days the plague bacillus lived on the various objects at different temperatures.*

| Temperature. | 37° C. | 22° to 29° C. | 17° to 19° C. |
|---|---|---|---|
| | *Days.* | *Days.* | *Days.* |
| Gelatin culture in albumin-gelatin balls | .......... | 75 | 112 |
| Agar suspension in— | | | |
| Tap water | 6 | 71 | 116 |
| Water | 6 | 71 | 96 |
| Bouillon culture on— | | | |
| Paper, dry | 0 | 3 | 8 |
| Paper, moist | .......... | 8 | 14 |
| Wood | 0 | 0 | 0 |
| Sawdust | 0 | 0 | 2 |
| Wool | .......... | .......... | 52 |
| Flannel | 3 | 3 | 3 |
| Sponge, dry | 2 | 97 | 125 |
| Garden earth, dry | 1 | 1 | 1 |
| Garden earth, moist | 14 | 84 | 93 |
| Bone | 1 | 6 | 17 |
| Agar suspension on bone dust— | | | |
| Dry | 1 | 4 | 6 |
| Moist | 125 | 108 | 125 |
| Bouillon culture on crash | 0 | 8 | 97 |
| Cultures on carpet and plush | 0 | 0 | 0 |
| Milk inoculated with plague | 49 | 68 | 32 |
| Bouillon culture in— | | | |
| Cheese | 17 | 17 | 13 |
| Butter | 5 | 55 | 72 |
| Cultures on figs and raisins | 0 | 0 | 0 |
| Bouillon culture on— | | | |
| Salt beef | .......... | 3 | .......... |
| Orange peel | .......... | 0 | .......... |
| Rice | 0 | 3 | 18 |
| Large variety of Chinese food products | .......... | .......... | 3 |
| Spleen of plague rabbit | 45 | 1 | 65 |
| Plague blood on— | | | |
| Filter paper | 0 | 4 | 6 |
| Crash | 0 | 4 | 1 |
| Sponge | 0 | .......... | 9 |
| Wood | 0 | .......... | 6 |

### No. 1.—Plague Cultures in Albumin-Gelatin Balls.

A gelatin culture of plague was mixed up with about an equal quantity of egg albumin and the mixture soaked on little balls of sterilized absorbent cotton about the size of a pea. These balls soon dried out and shrunk to hard, dry, flaky scales.

One-half was exposed in a Petri dish in the cool chamber at 17° to 19° C., and the other half in a dark room at 20° to 29° C. From time to time one of the dried balls was taken out and planted into bouillon. In case a growth appeared it was tested on media and animals.

*Plague cultures on gelatin-albumin balls.*

| Time. | Cold chamber, 17° to 19° C. | Dark room, 22° to 29° C. |
|---|---|---|
| 60 days | Growth<br>Virulent for mice in 3 and 4 days | Growth.<br>Virulent for mice in 4 and 5 days. |
| 75 days | Growth<br>Virulent for mouse in 5 days | Delayed growth.<br>Virulent for mouse in 2 days. |
| 112 days<br>Material expended. | Growth<br>Virulent for white rat in 3 days<br>Virulent for mouse in 3 days | No growth. |

### No. 2.—Plague Culture on Albumin Balls.

Little balls of sterilized absorbent cotton, about the size of a pea, were saturated with a 4-days-old culture of plague mixed with some egg albumin, as in No. 1, but omitting the gelatin.

| Cold chamber, 17° to 19° C. | | Remarks. | Dark room, 22° to 29° C. | | Remarks. |
|---|---|---|---|---|---|
| 18 days | + | | 19 days | + | |
| 22 days | + | | 22 days | + | |
| 42 days | + | | 42 days | + | |
| 59 days | + | | 59 days | — | |
| 68 days | + | | 68 days | — | |
| 78 days | — | Contaminated. | 78 days | — | Contaminated. |
| 89 days | — | | | | |

Note.—Throughout this paper + means growth and — no growth.

It is plain from these two series, Nos. 1 and 2, that plague will live a long time, even though dry, in an albuminous medium. In the one case it remained alive and virulent one hundred and twelve days, when the material was expended.

The influence of a few degrees of temperature upon the organism is prettily shown in these two series, for it lived thirty-seven days longer in one instance and twenty-six days longer in the other, although all the conditions were exactly the same, excepting a difference of 10° C. in the temperature.

## PLAGUE CULTURE IN TAP WATER.

The culture was taken from an agar slant and emulsified in a little bouillon. Four drops of this suspension were added to 15 c. c. of sterile tap water and kept under the conditions as stated below. From time to time a small quantity of the infected water was drawn off and planted for growth, and its virulence tested upon animals.

No. 3.—Plague Culture in Tap Water, 17° to 19 C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | + | Delayed growth. |
| 17 days | – | Small amount of water used. |
| 20 days | + | 2 c. c. used. |
| 25 days | + | Killed mouse in 3 days. |
| 34 days | + | |
| 45 days | + | |
| 66 days | + | Killed mouse in 5 days. |
| 80 days | + | |
| 96 days | + | Failed to kill mouse. |
| 116 days | + | |
| Material expended. | ........ | |

No. 4.—Plague Culture in Tap Water, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | + | Delayed growth. |
| 16 days | + | Contamination. |
| 20 days | + | Killed mouse in 24 hours. |
| 25 days | + | |
| 44 days | + | |
| 55 days | + | |
| 71 days | + | Killed mouse in 3 days. |
| 96 days | – | |
| 107 days | – | |
| Material expended. | | |

No. 5.—Plague Culture in Tap Water, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | – | |
| 17 days | – | |
| 20 days | – | 2 c. c. of the water inoculated into bouillon. |
| 24 days | - | |

This series (Nos. 3, 4, and 5) of plague in water was repeated, using distilled instead of tap water. But, as the suspensions were made in bouillon, and four drops of bouillon were added to each test tube containing 15 c. c. of water, the results can not be taken as showing the longevity of the bacillus in distilled water.

### No. 6.—Plague Culture in Water, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | + | Delayed growth. |
| 17 days | - | Small amount of water used. |
| 20 days | + | 2 c.c. used to inoculate into bouillon. |
| 25 days | + | Killed mouse in 5 days. |
| 34 days | + | |
| 45 days | + | |
| 66 days | + | Killed mouse in 3 days. |
| 80 days | + | |
| 96 days | + | Failed to kill mouse. |
| 116 days | - | Contaminated. |
| Material expended. | | |

### No. 7.—Plague Culture in Water, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | – | Contaminated. |
| 16 days | - | Do. |
| 20 days | + | 2 c. c. of the water inoculated into bouillon. |
| 25 days | + | Killed a mouse in 9 days. |
| 34 days | + | |
| 45 days | + | Failed to kill mouse. |
| 71 days | + | |
| 96 days | – | |
| 107 days | – | |
| Material expended. | | |

### No. 8.—Plague Culture in Water, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | – | |
| 17 days | – | |
| 20 days | – | |
| 24 days | – | |

These results plainly show that the plague bacillus will live a long time in water containing a trace of albuminous matter—there were four drops of bouillon to 15 c. c. of water.

The influence of temperature is very marked in these tests, for the organism lived only six days at 37° C., whereas it lived one hundred and sixteen days at 17° to 19° C. It will also be noticed that it lived longer at 17° to 19° than at 22° to 29° C. That is, the cooler the temperature the better does the organism resist.

It is of special importance to notice that the bacillus sometimes lost its virulence in water before it lost its power of vegetation.

### PLAGUE ON PAPER.

It was found that plague will not live very long on paper, even when in the presence of moisture. It usually died out in a few days, excepting when kept cool at 17° to 19° C., at which temperature it remained alive eight and fourteen days. It would appear from these experiments that letter mail is not apt to carry the infection, especially in hot weather.

Small slips of white filter paper were inoculated with a three-day-old bouillon culture of the bacillus pestis and placed in a Petri dish under the following conditions:

No. 9.—Bouillon Culture on Paper, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | − | |
| 4 days | − | |
| 6 days | − | |
| 8 days | − | |
| 10 days | − | |
| 12 days | − | Contaminated with a sarcina. |
| 14 days | − | |

No. 10.—Bouillon Culture on Paper, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | − | |
| 4 days | − | Contaminated with a long rod. |
| 6 days | − | |
| 8 days | − | |
| 10 days | − | |
| 12 days | − | |
| 14 days | − | |

No. 11.—Bouillon Culture on Filter Paper, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | The slips are dry. Delayed growth. |
| 8 days | + | |
| 14 days | − | Contaminated with a mould. |
| 25 days | − | |

### No. 12.—Bouillon Culture on Filter Paper, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | Delayed growth. Pure culture. |
| 8 days | – | |
| 14 days | – | |
| 25 days | – | |
| 35 days | – | |
| 46 days | – | |

In the three following experiments (Nos. 13, 14, and 15) a pledget of cotton soaked with sterile water was kept in the petri dish in order to see the effect of a moist atmosphere. Despite this fact the slips dried out quickly, but the organism lived a little longer than when allowed to dry without the cotton pledget, as in Nos. 9, 10, 11, and 12.

### No. 13.—Bouillon Culture on Filter Paper, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | Delayed growth. |
| 8 days | + | The slips are quite dry, despite the cotton pledget soaked with water. |
| 14 days | + | |
| 25 days | – | |
| 35 days | – | |
| 46 days | – | |
| 60 days | – | |

### No. 14.—Bouillon Culture on Filter Paper, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | Killed mouse in 18 hours. |
| 4 days | – | |
| 6 days | – | |
| 8 days | – | |
| 10 days | – | |
| 12 days | – | |
| 14 days | – | |

### No. 15.—Bouillon Culture on Filter Paper, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | + | |
| 25 days | – | |
| 35 days | – | |
| 46 days | – | |
| 60 days | – | |

## PLAGUE ON WOOD.

Plague could not be kept alive on pine wood, as the following tests will show.

Little splinters of white pine were sterilized by heat and soaked in a three-day-old bouillon culture of plague, then placed in petri dishes and exposed under the following conditions:

No. 16.—Bouillon Culture on Pine Wood, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | — | |
| 4 days | — | |
| 6 days | — | |
| 8 days | — | |
| 10 days | — | |
| 12 days | — | |
| 14 days | — | |

No. 17.—Bouillon Culture on Pine Wood, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | — | |
| 4 days | — | |
| 6 days | — | |
| 8 days | — | |
| 12 days | — | |
| 14 days | — | |

No. 18.—Bouillon Culture on Pine Wood, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | — | |
| 4 days | — | |
| 6 days | — | |
| 8 days | — | |
| 10 days | — | |
| 12 days | — | |
| 14 days | — | |

## PLAGUE CULTURE ON SAWDUST.

Sawdust was sterilized and saturated with a four-day-old culture of plague, and kept in a test tube stopped with cotton, so as to permit drying, under the following conditions:

One tube in the incubator at 37° C.

One tube in the cold chamber at 17° to 19° C.

One tube in a dark room at 22° to 29° C.

### No. 19.—Sawdust, in the Incubator, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | — | |
| 10 days | — | |
| 20 days | — | |
| 31 days | — | |

### No. 20.—Sawdust, in the Cold Room, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 10 days | — | |
| 20 days | — | |
| 30 days | — | |

### No. 21.—Sawdust, in a Dark Room, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | — | |
| 10 days | — | |
| 20 days | — | |

These results on sawdust correspond with other attempts made to keep the organism alive on wood. In no case was it found alive on wood after a few days.

## PLAGUE CULTURE ON WOOL.

Wool was inoculated with a culture of plague and kept in a Petri dish to dry out.

The following is very suggestive, for it indicates that the organism may remain alive a long time in the fur of animals:

### No. 22.—Plague on Wool.

| Time. | Result. | Remarks. |
|---|---|---|
| 4 days | + | |
| 7 days | + | |
| 27 days | + | The wool is dry. |
| 43 days | + | |
| 52 days | + | Contaminated. |
| 62 days | — | Do. |
| 73 days | — | Do. |
| 93 days | — | Do. |

## PLAGUE CULTURE ON FLANNEL.

Plague does not live long on flannel.

Small pieces of flannel were sterilized and saturated with a four-day-old bouillon culture of plague and left to dry under various temperatures.

No. 23.—Plague on Flannel, Dark Room, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 9 days | − | |
| 19 days | − | |

No. 24.—Plague on Flannel, Cold Chamber, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | − | |
| 19 days | − | |

No. 25.—Plague on Flannel, Incubator, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 9 days | − | |
| 19 days | − | |

## PLAGUE ON SPONGE.

Plague was kept alive a long time on sponge, in one instance one hundred and twenty-five days at 17° to 19° C. However, it lost its virulence for mice long before that time. In one instance the organism retained both its vitality and its virulence eighty days.

Small squares of sponge were sterilized and soaked with a three-day bouillon culture of plague. They were allowed to dry under the usual conditions in a Petri dish, as stated in the following tables:

No. 26.—Plague Culture on Sponge, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 4 days | + | |
| 6 days | + | |
| 8 days | + | |
| 12 days | + | Contaminated. |
| 14 days | + | |
| 21 days | + | |
| 31 days | + | |
| 43 days | + | Failed to kill a mouse; pieces of sponge very dry. |
| 52 days | + | |
| 63 days | + | Failed to kill a mouse. |
| 74 days | + | |
| 88 days | + | Failed to kill a mouse. |
| 98 days | + | |
| 125 days | + | |
| 134 days | − | |
| 144 days | − | |

No. 27.—Plague Culture on Sponge, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 4 days | + | |
| 6 days | + | |
| 8 days | + | Killed mouse in 9 days of plague. |
| 12 days | + | |
| 14 days | + | |
| 21 days | + | |
| 31 days | + | |
| 43 days | + | |
| 52 days | + | Contaminated. |
| 63 days | + | Failed to kill mouse. |
| 76 days | − | |
| 85 days | − | |
| 95 days | − | |

No. 28.—Plague Culture on Sponge, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 4 days | − | Contaminated. |
| 6 days | − | Do. |
| 8 days | − | Do. |
| 10 days | − | |
| 12 days | − | |
| 14 days | − | Do. |
| 21 days | − | Do |

These three experiments, as well as the following ones with sponge, show in a very definite manner the effect of temperature on the bacillus pestis. The organism lived only a few days in the incubator at the body temperature, while it survived over four months in the cold—17° to 19° C.

Another series of infected sponge was exposed in order to verify the rather remarkable results obtained in Nos. 26, 27, and 28. The results obtained were about the same, so it is plain that when well protected in a porous material like sponge the plague bacillus may live and retain its virulence a long time, even when dry.

No. 29.—Bouillon Culture on Sponge, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | + | |
| 14 days | + | |
| 25 days | + | The pieces of sponge are dry. |
| 35 days | + | |
| 46 days | + | |
| 60 days | + | |
| 70 days | + | |
| 80 days | + | 0.5 c. c. of a bouillon culture killed mouse in 2 days. |
| 97 days | – | |
| 106 days | – | |
| 116 days | – | |

No. 30.—Bouillon Culture on Sponge, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | + | |
| 14 days | + | |
| 25 days | + | Killed a mouse in 4 days. |
| 35 days | + | |
| 46 days | + | Contaminated. |
| 60 days | + | Failed to kill a mouse. |
| 70 days | + | |
| 80 days | + | |
| 97 days | + | |
| 106 days | – | |
| 116 days | – | |

Another series of experiments with sponge was tried to determine the effects of moisture. A pledget of absorbent cotton saturated with water was kept in the Petri dish along with the test objects. It was found difficult to prevent contamination in the presence of moisture, and also difficult to keep the objects moist so long a time.

No. 31.—Bouillon Culture on Sponge, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | + | Contaminated with a mould. |
| 14 days | + | |
| 25 days | + | |
| 35 days | + | |
| 46 days | + | Do. |
| 60 days | + | |
| 70 days | + | The pieces of sponge are dry |
| 80 days | + | Mould contamination. |
| 97 days | + | Do. |
| 106 days | — | |
| 116 days | — | |

No. 32.—Bouillon Culture on Sponge, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 8 days | + | |
| 14 days | — | |
| 25 days | — | |
| 35 days | — | The sponge is still moist. |
| 46 days | — | |
| 60 days | — | |

## PLAGUE ON GARDEN EARTH (ALLOWED TO DRY).

Garden earth was sterilized by heat and well mixed with a young bouillon culture of plague. This mixture was kept in a small bottle, loosely stoppered with a plug of cotton, in order to allow the earth to dry.

Three bottles so prepared were exposed under different conditions of temperature.

The organism did not live long under these conditions.

No. 33.—Bouillon Culture on Dry Garden Earth, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 11 days | + | |
| 17 days | — | |
| 25 days | — | Still moist. |

No. 34.—BOUILLON CULTURE ON DRY GARDEN EARTH, 22° TO 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | – | |
| 11 days | – | |
| 17 days | – | |
| 25 days | | |

No. 35.—BOUILLON CULTURE ON DRY GARDEN EARTH, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 4 days | – | The garden earth is dry. |
| 6 days | – | |
| 11 days | – | |
| 17 days | – | |
| 25 days | – | |

## PLAGUE ON GARDEN EARTH (KEPT MOIST).

The garden earth was sterilized and mixed with a bouillon culture of plague as in the preceding three experiments, the only difference being that the bottles were tightly closed with a cork stopper in order to keep the earth moist.

The bacillus lived a much longer time under these conditions, as was to be expected.

The influence of temperature is well shown here, for at 37° C. the bacillus died after fourteen days, while at cooler temperatures it lived eighty-four and ninety-three days, all the other conditions being precisely similar.

No. 36.—PLAGUE CULTURE ON MOIST GARDEN EARTH, 17° TO 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 11 days | + | |
| 22 days | + | |
| 32 days | + | |
| 46 days | + | |
| 56 days | + | Failed to kill a mouse. |
| 83 days | + | Contaminated. |
| 93 days | + | Do. |
| 102 days | – | Contaminated; still moist. |
| 113 days | | Contaminated; dry. |

No. 37.—Plague Culture on Moist Garden Earth, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 11 days | + | |
| 22 days | + | |
| 35 days | + | |
| 47 days | + | |
| 67 days | + | |
| 84 days | + | |
| 93 days | — | Still moist. |
| 103 days | — | |

No. 38.—Plague Culture on Moist Garden Earth, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 5 days | + | |
| 14 days | + | |
| 25 days | + | |
| 36 days | — | |
| 50 days | — | |
| 60 days | — | Still moist. |

## PLAGUE CULTURE ON BONE.

Plague will not live long on a piece of dry bone, for a bouillon culture saturated on small porous bits soon died out.

An old dried femur of an ox was broken into bits and sterilized by heat, then saturated with a three-day bouillon culture and exposed in the usual manner in a Petri dish, under different temperature conditions.

No. 39.—Bouillon Culture on Bone, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 11 days | — | |
| 17 days | + | Delayed growth; failed to kill mouse. |
| 25 days | — | |
| 34 days | — | |
| 45 days | — | |

No. 40.—Bouillon Culture on Bone, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | Bone is dry. |
| 11 days | — | |
| 17 days | — | |
| 25 days | — | |

### No. 41.—Bouillon Culture on Bone, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | — | The pieces of bone are dry. |
| 11 days | — | Contaminated with a mould. |
| 17 days | — | Do. |

## AGAR SUSPENSION ON BONE DUST.

The organism died very quickly when the infected bone dust was permitted to dry, in no case living more than six days. In marked contrast, it lived a very long time on the same bone dust when kept moist.

The bone dust came from the cargo of a vessel from Bombay. It was sterilized by heat and then saturated with an agar-agar suspension of plague in water and thoroughly mixed. The mixture was placed in small bottles and in the following three experiments loosely stoppered with cotton so as to permit drying.

### No. 42.—Bouillon Culture on Dry Bone Dust, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Loosely stoppered so as to dry. |
| 4 days | + | Killed mouse in 3 days. |
| 6 days | — | |
| 11 days | — | |
| 17 days | — | |
| 25 days | — | |

### No. 43.—Bouillon Culture on Dry Bone Dust, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Loosely stoppered so as to dry. |
| 4 days | + | The bone dust is dry. |
| 6 days | + | Killed mouse of plague in 18 hours. |
| 11 days | — | |
| 17 days | — | |
| 25 days | — | |

### No. 44.—Bouillon Culture on Dry Bone Dust, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Loosely stoppered so as to dry. |
| 4 days | — | The bone dust is dry. |
| 6 days | — | |
| 11 days | — | |
| 17 days | — | Contaminated with a mold. |
| 25 days | — | |

In the following three experiments the bottles were tightly corked, so as to keep the infected bone dust moist. All the other conditions were the same as the three preceding experiments (Nos. 42, 43, and 44).

It is remarkable that the plague bacillus lived so long a time in watery suspension on this material—one hundred and twenty-five days. It is especially to be noted how long the organism lived at 37° C., a temperature which in this work has almost universally proved fatal for the organism, even when kept moist on various objects.

### No. 45.—Agar Suspension on Moist Bone Dust, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Kept moist. |
| 3 days | + | |
| 5 days | + | |
| 7 days | + | |
| 13 days | + | |
| 20 days | + | |
| 30 days | + | |
| 43 days | + | Contaminated with a long rod. |
| 52 days | + | |
| 63 days | + | |
| 74 days | + | |
| 88 days | + | |
| 98 days | + | Failed to kill a mouse. |
| 108 days | + | Almost dry. |
| 125 days | + | Dry. |
| 134 days | – | |
| 144 days | – | Contaminated. |

### No. 46.—Agar Suspension on Moist Bone Dust, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Kept moist. |
| 3 days | + | |
| 5 days | + | |
| 7 days | + | |
| 14 days | + | |
| 31 days | + | |
| 43 days | + | Failed to kill a mouse. |
| 52 days | + | Do. |
| 63 days | + | Mould contamination. |
| 76 days | + | |
| 88 days | + | The bone dust is still moist. |
| 108 days | + | The bone dust is now dry. |
| 125 days | – | |
| 134 days | – | |

No. 47.—AGAR SUSPENSION ON MOIST BONE DUST, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | Kept moist. |
| 3 days | + | |
| 5 days | + | |
| 7 days | + | |
| 14 days | + | |
| 21 days | + | |
| 31 days | + | |
| 43 days | + | Killed a mouse in 8 days. |
| 52 days | + | Contaminated with a mold. |
| 63 days | + | Failed to kill a mouse. |
| 74 days | + | |
| 88 days | + | |
| 98 days | + | The bone dust is still moist. |
| 109 days | + | The bone dust is drying out. |
| 125 days | + | The bone dust is dry. |
| 131 days | – | Contaminated. |
| 141 days | – | |

## PLAGUE ON CRASH.

As a rule, plague dies very quickly on crash, which would indicate that fabrics are not very apt to carry the infection.

Usually it died in a few days, although in one instance it was found to be alive on a piece of crash ninety-seven days from the time it was inoculated. This plainly shows that there are factors in the viability of this organism that are but imperfectly understood which occasionally preserve its vitality.

The small squares of crash were sterilized and saturated with a three-day broth culture of plague and allowed to dry in Petri dishes.

No. 48.—BOUILLON CULTURE ON CRASH, 17° TO 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | – | |
| 4 days | – | |
| 6 days | – | |
| 8 days | – | |
| 10 days | – | |
| 12 days | – | |
| 14 days | – | |

No. 49.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | – | |
| 4 days | – | |
| 6 days | – | |
| 8 days | – | |
| 10 days | – | |
| 12 days | – | |
| 14 days | – | |

### No. 50.—BOUILLON CULTURE ON CRASH, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days .............. | – | |
| 4 days .............. | – | |
| 6 days .............. | – | |
| 8 days .............. | – | |
| 10 days .............. | – | |
| 12 days .............. | – | |
| 14 days .............. | – | |

### No. 51.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days .............. | + | |
| 8 days .............. | + | |
| 14 days .............. | – | Contaminated. |
| 25 days .............. | – | |
| 35 days .............. | – | |
| 46 days .............. | – | |
| 60 days .............. | – | The crash is still moist. |

### No. 52.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days .............. | – | |
| 5 days .............. | – | |
| 8 days .............. | – | |
| 14 days .............. | – | |

### No. 53.—BOUILLON CULTURE ON CRASH, 17° TO 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days .............. | + | |
| 8 days .............. | + | |
| 14 days .............. | + | Mould contamination. |
| 25 days .............. | – | Do. |
| 35 days .............. | – | Mould contamination. The crash still moist. |
| 46 days .............. | – | |
| 60 days .............. | – | |

No. 54.—Bouillon Culture on Crash, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | Permitted to dry in Petri dish. |
| 8 days | + | |
| 14 days | + | |
| 25 days | + | Mould contamination. |
| 35 days | + | Failed to kill mouse. |
| 46 days | + | |
| 60 days | + | |
| 70 days | + | |
| 80 days | + | Contaminated. |
| 97 days | + | |
| 106 days | – | Do. |
| 116 days | – | Do. |

### PLAGUE ON CARPET AND PLUSH.

Plague could not be made to live on carpet and plush. Many samples of carpet and plush were sterilized and inoculated with plague, but from none was the organism recovered. It is possible that the dyestuff has something to do with this result, for all the samples used had soluble dyes. Plague could be grown in the solution of the dyes, so that the dyes did not possess the power of preventing growth. It is not evident why plague should not live in carpet and plush as well as it does on crash and paper, and the results emphasize the fact that there are factors that influence the viability of the germ that are not well understood.

### PLAGUE IN MILK.

According to all reported works upon the subject, and the three tests that follow, milk is a good medium for plague.

Three test tubes of milk were sterilized and inoculated with a two-day-old agar culture of plague, one loop of culture used for each tube.

The three tubes were incubated at 37° C. for one day, then placed under different conditions:

One left in the incubator at 37° C.

One left in the cold chamber at 17° to 19° C.

One left in a dark room at 22° to 29° C.

From time to time small quantities of the milk were transferred to bouillon for growth. The milk was contaminated, which contamination made the identification of the plague bacillus more difficult and doubtless influenced its longevity.

### No. 55.—Milk, in the Incubator at 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 9 days | (?) | Contaminated. |
| 12 days | (?) | Do. |
| 22 days | + | Killed mouse of plague in 3 days. |
| 33 days | + | |
| 49 days | + | |
| 58 days | – | Contaminated. |
| 68 days | – | Do. |

### No. 56.—Milk, in the Cold Chamber at 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 8 days | + | Contaminated. |
| 22 days | + | |
| 32 days | + | Failed to kill mouse. |
| 49 days | – | Contaminated. |
| 58 days | – | Do. |
| 68 days | – | Do. |

### No. 57.—Plague in Milk, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 9 days | + | |
| 22 days | + | |
| 49 days | + | |
| 58 days | – | No growth of any kind. |
| 68 days | + | Pure and typical plague. |
| 79 days | – | No growth from larger amount of milk inoculated. |
| 99 days | – | |

## PLAGUE CULTURE IN CHEESE.

The organism lived seventeen days in cheese.

American cheese was melted in a water bath, poured into test tubes, and sterilized by the fractional method at low temperatures.

Four cubic centimeters of a three-day-old growth of plague in bouillon was thoroughly mixed up with 15 c. c. of cheese in each of three tubes. These tubes were then exposed under different conditions of temperature.

(1) In the incubator at 37° C.
(2) In the cold chamber at 17° to 19° C.
(3) In a dark room at 22° to 29° C.

### No. 58.—PLAGUE IN CHEESE, IN THE INCUBATOR, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 6 days | + | |
| 13 days | + | |
| 17 days | + | |
| 26 days | – | Growth. |
| 36 days | – | Subtilis contamination. |

### No. 59.—PLAGUE IN CHEESE, IN THE COLD CHAMBER, 17° TO 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 6 days | + | |
| 13 days | + | |
| 26 days | – | No growth. |
| 36 days | – | Do. |
| 47 days | – | Do. |
| 67 days | – | Do. |

### No. 60.—PLAGUE IN CHEESE, IN A DARK ROOM, 22° TO 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 6 days | + | |
| 13 days | + | |
| 17 days | + | |
| 26 days | – | No growth. |
| 36 days | – | Contaminated with a spor-bearing rod. |
| 47 days | – | Do. |

## PLAGUE IN BUTTER.

Plague may live a long time in butter, especially when kept cool—that is, at 17° to 19° C. The warmer the temperature the shorter the time the organism lives. At 37° C. it remained alive but five days, against seventy-two days at 17° to 19° C.

Butter was melted and sterilized in test tubes at 100° C. for a few minutes each of three days. While liquid it was inoculated with a three-day-old broth culture of plague. Two cubic centimeters of the culture was mixed in each test tube, holding about 15 c. c. of butter.

These tubes were kept under three conditions of temperature:

(1) In the incubator at 37° C.

(2) In the cold chamber at 17° to 19° C.

(3) In a dark room at 22° to 29° C.

From time to time small amounts were taken from each tube and planted in bouillon.

No. 61.—Plague Culture in Butter, in the Incubator, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 5 days | + | Killed mouse in 5 days. |
| 14 days | – | |
| 24 days | | |

No. 62.—Plague Culture in Butter, in Cold Chamber, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 14 days | + | |
| 24 days | + | Killed mouse in 3 days. |
| 53 days | + | |
| 63 days | + | |
| 72 days | + | |
| 82 days | – | |
| 91 days | – | |

No. 63.—Plague Culture in Butter, in a Dark Room, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 5 days | + | |
| 14 days | + | |
| 24 days | + | Killed mouse in three days. |
| 35 days | + | |
| 55 days | + | |
| 65 days | – | |
| 71 days | – | |

## PLAGUE ON FIGS AND RAISINS.

Raisins and figs were contaminated with cultures of plague, and the attempts made from time to time failed to recover the organism by means of cultures and inoculations. This work was undertaken in order to determine whether such articles from infected localities could transmit the infection. The difficulties of determining this question were increased by the fact that it is not practicable to sterilize the figs and raisins. It was also found that both these articles contain 58 per cent of glucose, and that the plague bacillus will not grow in a 58 per cent glucose medium.

The plague organism was never recovered from raisins and figs, even on the day following the inoculation, and it may be definitely stated that there is little danger of these articles carrying the infection.

## PLAGUE CULTURE ON SALT BEEF.

Salt beef was sterilized and saturated with a four-day-old broth culture of plague and kept in a dark room at 22° to 29° C.:

### No. 64.—Bouillon Culture in Salt Beef, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | Contaminated. |
| 18 days | — | No growth. |
| 28 days | — | Subtilis. |

## PLAGUE CULTURE ON ORANGE PEEL.

Orange peel was sterilized and saturated with a four-day-old broth culture of plague and kept in a dark room at 22° to 29° C.:

### No. 65.—Bouillon Culture in Orange Peel, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | — | |
| 18 days | — | |

## PLAGUE ON RICE.

Rice was sterilized and inoculated with a four-day-old bouillon culture of plague and kept under the following conditions:

### No. 66.—Plague on Rice, in Dark Room, 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 18 days | — | |
| 28 days | — | |

### No. 67.—Plague on Rice, Cold Chamber, 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | + | |
| 18 days | + | |
| 28 days | — | |
| 38 days | — | |

### No. 68.—Plague on Rice, in Incubator, 37° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 3 days | — | |
| 18 days | — | Contaminated. |

These results correspond with those of Hankin, who, following different methods, found that plague lived but a short time (few days) on grain.

### PLAGUE BLOOD AND TISSUES.

Plague may live a considerable time in the tissues and organs of animals dead of that disease, when these substances are preserved under special conditions. It is probable that the saprophrytes, which soon invade the organism of animals buried or disposed of in the usual way, soon overpower and determine the death of the plague organism. In this work, when the plague bacillus could be protected from such influences, it was found to remain alive and virulent for sixty-five days in a piece of dried spleen.

The spleen of a rabbit dead of experimental plague was placed with aseptic precautions in a petri dish and kept in the cold chamber at 17° to 19° C. The spleen soon dried out. Pieces were taken from time to time and planted in bouillon.

No. 69.—Spleen of Plague Rabbit.

| Time. | Result. | Remarks. |
|---|---|---|
| 6 days | + | |
| 18 days | + | The spleen is very dry. |
| 20 days | + | |
| 34 days | + | |
| 43 days | + | |
| 65 days | + | Killed mouse in 6 days. |
| Material expended | | |

No. 70.—Spleen of Plague Rabbit.

The spleen of a rabbit dead of experimental plague was placed in a Petri dish, but first the surface was sterilized by moistening and burning with alcohol, and kept in a dark room at 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 13 days | — | |
| 27 days | — | |

### No. 71.—Spleen of Plague Rabbit.

The entire spleen of a rabbit dead of experimental plague was kept in a Petri dish in the incubator at 37° C. The spleen soon dried out and became hard and friable. Small pieces were taken from time to time and planted into bouillon.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 12 days | + | Killed mouse of plague in 18 hours. |
| 26 days | + | |
| 35 days | + | Contaminated. |
| 45 days | + | Failed to kill mouse. |
| Material expended. | | |

## PLAGUE BLOOD ON VARIOUS OBJECTS.

Plague-infected blood from animals dead of septicæmic plague was inoculated upon various objects, such as paper, wood, crash, sponge, etc., and kept under various conditions of temperature.

It was found that such thin, dry layers of blood will not preserve the bacillus very long—at most nine days.

### No. 72.—Plague Blood on Filter Paper.

The heart's blood of a rabbit dead of experimental plague was inoculated on little slips of white filter paper. These slips were placed in a Petri dish and allowed to dry out in a dark room at 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | Killed rabbit over night by intravenous inoculation. |
| 4 days | + | |
| 6 days | – | |
| 13 days | – | |
| 25 days | – | |

### No. 73.—Plague Blood on Crash.

The heart's blood of a rabbit dead of experimental plague was inoculated on little squares of sterile fabric (crash) and then allowed to dry out in a Petri dish exposed in a dark room at 22° to 29° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 2 days | + | |
| 4 days | + | |
| 6 days | – | |
| 13 days | – | |
| 25 days | – | |

### No. 74.—Plague Blood on Sponge.

Small pieces of sponge were sterilized by heat and impregnated with the heart's blood of a rabbit dead of experimental plague and kept in a Petri dish in the cold room at 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day ................ | + | |
| 6 days ............... | + | |
| 9 days ............... | + | |
| 12 days .............. | – | |
| 18 days .............. | – | Contaminated. |
| 31 days .............. | – | |

### No. 75.—Plague Blood on Crash.

Small squares of crash were impregnated with the heart's blood of a rabbit dead of experimental plague and kept in the cold room at 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day ................ | + | Contaminated. |
| 6 days ............... | – | |
| 9 days ............... | – | |
| 12 days .............. | – | |
| 18 days .............. | – | |

### No. 76.—Plague Blood on Paper.

Small slips of sterilized white filter paper were impregnated with several drops of the heart's blood of a rabbit dead of experimental plague and exposed in the cold room at 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day ................ | + | |
| 6 days ............... | + | |
| 9 days ............... | – | |
| 12 days .............. | – | |
| 18 days .............. | – | |
| 34 days .............. | – | |

### No. 77.—Plague Blood on Wood.

Small sticks of white pine were sterilized by heat and soaked in the heart's blood of a rabbit dead of experimental plague and left to dry in a Petri dish in the cold room at 17° to 19° C.

| Time. | Result. | Remarks. |
|---|---|---|
| 1 day | + | |
| 6 days | + | |
| 9 days | — | |
| 12 days | — | |
| 18 days | — | |

### Plague Blood on Crash, Paper, Sponge, and Wood at 37° C.

Small pieces of crash, white filter paper, sponge, and white pine were sterilized and soaked in the heart's blood of a rabbit dead of experimental plague and kept in the incubator at 37° C.

NO. 78.

| Object. | Time. | Result. |
|---|---|---|
| Crash | 5 days | — |
| Do | 14 days | — |
| NO. 79. | | |
| Paper | 5 days | — |
| Do | 14 days | — |
| NO. 80. | | |
| Sponge | 5 days | — |
| Do | 14 days | — |
| NO. 81. | | |
| Wood | 5 days | — |
| Do | 14 days | — |

### PLAGUE EXPOSED TO SUNLIGHT.

Plague when exposed directly to bright sunlight died quickly. Even in a piece of sponge it could not live an hour in the bright sunlight of a Washington day in the month of May. When exposed more directly on the surface of objects it died within half an hour.

The sun, however, could not penetrate small pieces of plague spleen in five hours.

### No. 82.—Plague Cultures on Various Objects Exposed to the Sunlight.

A two-day-old bouillon culture was reenforced with an oese of an agar culture, and then bits of sterilized paper, crash, sponge, and wood were saturated with the mixture and exposed in a Petri dish to direct sunlight. The temperature in the sun at the time was 33.2° to 35.5° C. This was on May 14, in Washington.

*Effects of sunlight.*

| Object. | Hours. | | | | | |
|---|---|---|---|---|---|---|
| | ½ | 1 | 2 | 3 | 4 | 6 |
| Filter paper | – | – | – | – | – | – |
| Crash | – | – | – | – | – | – |
| Sponge | + | – | – | – | – | – |
| Wood | – | – | – | – | – | – |

### No. 83.—Spleen of Plague Rabbit Exposed to Sunlight.

A small piece of the spleen of a rabbit dead of experimental plague was exposed in a Petri dish to bright sunlight, May 26. At the end of five hours it was crushed and planted into broth and gave a typical growth, which growth inoculated into a mouse killed it in three days. The plague organism recovered in pure culture from blood and spleen.

## CONCLUSIONS.

(1) The bacillus pestis is not a frail organism. It resembles the hemorrhagic septicæmic group or the cocco-bacilli as far as its viability is concerned.

(2) Temperature is the most important factor in the viability of the plague bacillus. It keeps alive in the cold, under 19° C., a very long time. It dies quickly, especially when dried, at the body temperature, 37° C.

(3) Moisture favors the life of the bacillus pestis. It usually dies in a few days when dry, even in the presence of albuminous matter, provided the temperature is above 30° C. It may keep alive and virulent when dry for months in the cold, under 19° C.

(4) Sunlight kills the organism within a few hours, provided the sun shines directly upon the organism and the temperature in the sun is over 30° C. The effect of sunlight is not very penetrating.

(5) The virulence of the bacillus pestis is often lost before its vegetability.

(6) It is unlikely that new dry merchandise would carry the infection. The organism usually dies in a few days on the surface of objects such as wood, sawdust, bone, paper, etc.

(7) Clothing and bedding can harbor the infection for a long time

and may act as fomites. The bacillus lives for months when dry in albuminous media at temperatures under 20° C.

(8) Food products may carry the infection of plague. The bacillus lives a long time in milk, cheese, and butter. It usually dies quickly on the surface of fruits and prepared foods.

(9) The organism may live a long time in water, although plague is not a water-borne disease.

(10) The plague bacillus does not live long on paper, and first-class mail is therefore not apt to convey the infection.

(11) The colder the climate the greater the danger of conveying the infection on fomites—clothing, bedding, food, merchandise, etc.—and more extensive disinfection is required in such a climate in combating the disease than in tropical regions.

(12) The plague bacillus is destroyed by sulphur fumigation and by formaldehyde gas in the strengths in which these disinfectants are usually employed. The gases can only be depended upon as surface disinfectants. In disinfecting ships, warehouses, dwellings, and other places infested with rats, fleas, and vermin, sulphur is better than formaldehyde, because formaldehyde gas fails to kill the higher forms of animal life.

(13) A temperature of 70° C. continued a short time is invariably fatal for the plague bacillus. The ordinary antiseptics are all efficacious in their usual strength for nonspore-bearing organisms. Efficient surface disinfection may be accomplished by exposing objects all day to the direct sunshine on warm days. The temperature in the sun must be above 30° C.

# PLAGUE ON VARIOUS FOOD STUFFS.

[By Passed Assistant Surgeon H. D. Geddings, Marine-Hospital Service.]

JANUARY 11, 1901.

The experiments were continued from the point where Dr. Rosenau's preliminary note left off, and have been the subject of continuous observations in this laboratory for more than a year from that time, having only been brought to a conclusion within the past sixty days.

In general terms it may be said that the further observations have simply confirmed his preliminary views. The growth of the bacillus continued, reaching under various conditions of temperature and moisture to a period of one hundred and twenty-five days on bone dust, where moisture was preserved, and at a temperature of 37° C.; ninety-seven days on crash, temperature 22° to 29° C.; ninety-six days in distilled water, temperature 17° to 19° C.; ninety-seven days in tap water, and other periods too numerous here to mention.

But I would beg here to remind you that while these results are entirely beyond question, and while no effort was spared to make the conditions simulate those naturally obtaining, there were in the end some decided departures from the normal which would obtain under conditions of commerce, trade, etc.

The experiments all showed, too, that under conditions of dryness, exposure to sunlight, and to temperatures above the normal, but not very elevated, the life of the bacillus was short.

Again, too, I would invite your attention to the fact that a condition which obtained in all these experiments was one very far from obtaining under normal conditions, viz, the fact of an intentional, abundant inoculation with a pure culture of the materials subjected to test, and this culture one of known activity and virulence.

A second departure from normal conditions was in the fact that all the materials receiving the abundant inoculation of the virulent pure culture were sterile. In other words, the plague bacillus was not only put to growing under the before-mentioned favorable conditions of heat and moisture, but was relieved in toto from that microbial symbiosis which, in the economy of nature, plays so important a part in the suppression of pathogenic organisms.

I do not think, therefore, that the laboratory experiments, complete and painstaking though they be, should be held as establishing a standard in the case of food products where the following conditions would establish a strong dissimilarity of conditions:

First. Food products are never intentionally inoculated with plague organisms, or, for that matter, with any others. On the contrary, no matter how careless the operator and how slovenly the method of preparation, care is exercised to protect from gross contamination. The product, it is true, may accidentally become contaminated by preparation in a dwelling where plague prevails. After preparation it may become contaminated in the warehouse or store by being "muzzled" by a rat suffering from the pneumonic form of the disease, or a plague-infected rat may nest or even die on the products, or the foods may be contaminated by dust containing virulent and viable plague bacilli.

But granting any or all of these possibilities, should the contamination take place it is at once confronted by the following condition:

Second. That all products for wear, for use in the arts, or for food are the seat of contamination by saprophytic bacteria, and that there is at once set up a conflict between the pathogene on the one hand and the saprophyte on the other, in which contests the hardier saprophyte usually comes off victorious over the more deadly but more highly organized and sensitive pathogene.

I repeat, therefore, that the conditions obtaining in exact laboratory experimentation are not applicable to and do not form an invariable rule of conduct for quarantine matters, in which more normal natural conditions obtain.

The continuation of the experiments which formed the basis for Dr. Rosenau's preliminary note lead in the main to the conclusion that if a substance is favorable for the retention of moisture, and a comparatively low temperature (17° to 19° C.), it will afford a good culture medium for the growth of the plague bacillus, unless it have in itself some inherent property which renders it unsuitable. This latter may be something added to it in the process of manufacture, as the sizing or filling in certain fabrics, or the resins or gums contained in woods, or the bacteria which find a habitat in manufactured foods.

Thus the plague bacillus failed to grow on linen crash until it had been thoroughly freed of its sizing by washing; on sawdust it lived but one day; on flannel only three days; on a piece of carpet (the effect probably of the dye) it did not grow at all.

The following food products were tried, and gave the following results: On cheese it lived from thirteen to seventeen days, but this only after the cheese had been thoroughly sterilized and melted; on rice, sterilized, three days at 22° to 29° C.; on dried salt beef, three days; on orange peel, no growth; on figs and raisins, no growth, though subsequent experiments showed this to be due to the amount of glucose or fruit sugar contained.

Further, a staple article of Chinese food, a duck dried and smoked, was obtained, and from this small pieces were cut and placed in sterile Petri dishes. These pieces were then liberally inoculated with a bouillon culture of the plague bacillus and the dishes placed in the incubator. Another portion was placed directly in a tube of bouillon, and its growth proved that it was free from bacteria, save the hay bacillus and its spores.

For several days small pieces were cut off from those contained in the dishes, and these were also planted into bouillon tubes. The length of life of the plague bacillus under these conditions and this microbial association is uncertain. For two or three days they were present in numbers; this number grew rapidly more limited, and by the twelfth day the plague bacilli were difficult of detection. Between this and the eighteenth day they disappeared completely, though the hay bacillus was still active and grew in great abundance.

Subsequently a large assortment of Chinese food products was obtained, and with these experiments were made. The products in question consisted of the legs of smoked and dried ducks; dried oysters; dried cuttlefish; dried ducks' gizzards; ducks' gizzards dried and then placed in oil; smoked and dried pork; duck eggs preserved in a mixture of mud and rice chaff. In this last the mixture had originally been wet, but had dried out until there only remained a mass of pulverulent earth and chaff surrounding the egg—in other words, dry earth.

Portions from each of these specimens were thoroughly inoculated with a bouillon culture of the plague bacillus, and the dishes containing them were placed in the incubator at 37° C. for about twenty-eight hours. Small pieces were then cut off each and these pieces planted into bouillon tubes, which were incubated for twenty-four hours.

Of the seven specimens, three alone showed any growth viz. the pork, the dried gizzard, and the cuttlefish.

A specimen from the growth in such case was removed and stained with carbol-thionin solution (the stain by election for plague bacilli), and microscopic examination showed the following: On the pork the growth was the B. subtilis (hay bacillus) alone; on the dried gizzard, hay bacillus; on the dried cuttlefish, hay bacillus and an ordinary mold.

In view of these results, therefore, I would respectfully submit the opinion that it is unlikely that the Chinese food products in question could convey the germ of plague, even should they accidentally become contaminated with the same, and under the ordinary conditions of commercial intercourse I much question whether such contamination is likely to occur, or whether in the presence of the temperature obtaining in the holds of ships the organism would have any prolonged life.

As these results may have a bearing on the quarantine restrictions at present in force against these food stuffs, they are submitted at once, without waiting for further observations, which, however, will be carried on from time to time.

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